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Spatial distribution and insecticide susceptibility profile of aedes aegypti mosquitoes in south-eastern, Tanzania

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SPATIAL DISTRIBUTION AND INSECTICIDE SUSCEPTIBILITY PROFILE OF *AEDES AEGYPTI* MOSQUITOES IN SOUTH-EASTERN, TANZANIA

Najat Kahamba

A dissertation submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Aedes-borne diseases such as dengue and chikungunya constitute constant threats globally. In Tanzania, the main vector species is *Aedes aegypti*, which is widely distributed in urban areas, but whose ecology remains poorly-understood in growing towns and secondary cities. We collected adult mosquitoes using Gravid *Aedes* trap and surveyed aquatic habitats of *Ae. aegypti* mosquitoes in and around Ifakara, a fast-growing town in south-eastern Tanzania. Field-collected mosquitoes were tested for susceptibility to common insecticides in dry and rainy seasons. A total of 926 mosquitoes were collected, 431 (46.5%) were identified as *Aedes aegypti*, 487 (52.5%) *Culex*, 8 (0.01%) as other *Aedes* and 13 (0.01%) as *Anopheles* mosquitoes. Of 1515 and 1933 aquatic habitats examined in dry and rainy seasons respectively, 18.87% and 14.64% contained *Aedes* immatures (container index. In the 2315 and 2832 houses visited in dry and rainy seasons, 4.9% and 6.6% had at least one *Aedes*-positive habitat. The main habitat types included: (a) used vehicle tires and discarded containers, (b) flower pots and clay pots, and (c) holes made by residents on trunks of coconut trees to support climbing harvesters. *Aedes aegypti* adults were susceptible to all tested insecticides in both seasons, except bendiocarb, against which resistance was observed in rainy season. The high infestation levels indicate significant risk of *Aedes*-borne diseases, requiring immediate action to prevent potential outbreaks in the area. While used tires, discarded containers and flower pots are key habitats for *Aedes*, this study also identified coconut harvesting as an important risk factor, and the associated tree-holes as potential targets for *Aedes* control. Since *Ae. aegypti* mosquitoes are still susceptible to insecticides, effective control could combine environmental management, preferably involving communities, habitat removal and insecticide spraying.

Keywords: *Aedes aegypti*, Habitat characterization, Insecticide susceptibility, Ifakara Health Institute, Dengue, Chikungunya, Tanzania

DECLARATION

I, Najat Kahamba do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation titled "Spatial distribution and insecticide susceptibility profile of *Aedes aegypti* mosquitoes in south-eastern, Tanzania" is my original work and has never been or intending to be submitted for a degree award in any other university.

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Najat Kahamba Date

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CERTIFICATION

This is to certify that I have read and approved the dissertation titled, "Spatial distribution and insecticide susceptibility profile of *Aedes aegypti* mosquitoes in south-eastern, Tanzania" submitted by Najat Kahamba (M394/ T.17) in partial fulfillment of the requirements for the award of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology**.**

Fredros Okumu, PhD
Adjunct Professor, NM-AIST
Supervisor

Date

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DEDICATION

I would like to dedicate this work to my beloved daughters Salma Salim Abubakar and Samreen Salim Abubakar.

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LIST OF ABBREVIATIONS AND SYMBOLS

BI	Breteaux Index
CDC	Centre of Control and Disease prevention
CI	Container Index
CIESIN	Centre for International Earth Science Information Network
ESRI, USA	Environmental Systems Research Institute, United State of America
GAT	Gravid *Aedes* Trap
GPS	Geographical Position System
GVCR	Global Vector Control Response
HI	House Index
HRSL	High Resolution Settlement Layer
IDW	Inverse Distance Weighted
IHI	Ifakara Health Institute
KDT	Knock Down Time
KDT_{50}	Time taken for 50% of mosquitoes to knock down
KDT_{95}	Time taken for 95% of mosquitoes to knock down
MRCC	Medical Research Coordinating Committee
NIMR	National Institutes of Medical Research
OR	Odds Ratios
RR	Relative Rare/Relative Risk
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

In recent decades, significant attention has been put on controlling mosquitoes that transmit malaria, leading to significant progress since 2000 (Bhatt *et al*., 2016; World Health Organization (WHO), 2018b). However, other mosquito-borne diseases, such as dengue, yellow fever, chikungunya and zika, which are transmitted by *Aedes* mosquitoes remain largely neglected. Golding *et al*. (2015) showed that more than 90% of persons at risk of vector-borne diseases are affected by at least two such diseases, malaria and dengue fever being the commonest (Golding *et al*., 2015). The WHO Global Vector Control Response (GVCR) initiative therefore recommended integrated approaches to address multiple vectors and vector-borne diseases (Golding *et al*., 2015). Unfortunately, unlike malaria, for which effective prevention and treatment options are widely available, the *Aedes*-borne diseases still rely mostly on personal protection measures (World Health Organization, 2003a), even though vaccine trials are increasingly advanced as well (Biswal *et al*., 2019).

In Tanzania, concerns about *Aedes*-borne diseases have become increasingly prominent in recent years due to multiple outbreaks, detection of the viruses in humans, and the wide distribution of the *Aedes* mosquitoes (Chipwaza *et al*., 2014; Hertz *et al*., 2012; Kajeguka *et al*., 2016; Patrick *et al*., 2018). Dengue cases have been reported in multiple regions in the country, including Dar es Salaam city, the islands of Zanzibar and Pemba, Mbeya and Iringa areas in southern Tanzania, and Kilimanjaro in the north (Mboera *et al*., 2016; Vairo *et al*., 2012). The most recent outbreak occurred in May 2019, when 1012 new cases were confirmed over just two weeks (World Health Organization, 2018a). By September 2019, 6912 cases had been reported, including 13 deaths (World Health Organization, 2018a).

Most outbreaks of *Aedes*-borne diseases have been observed in urban areas, where densities of both the vector and humans are high (Mboera *et al*., 2016). However, human mobility has also led to introduction of viruses in rural areas and small towns (Chipwaza *et al*., 2014). Unfortunately efforts against these diseases are hampered by lack of proper medication or diagnostics (Simmons

et al., 2015; Stoler *et al*., 2014; Wiwanitkit, 2010). Effective vector surveillance and control to prevent potentially-infectious mosquito bites therefore remain core components of programs targeting such diseases (World Health Organization, 2003a).

1.2 Statement of the problem

Current understanding of *Aedes* mosquitoes is largely studied in urban areas where the vector is most widespread (Bataille *et al*., 2010). *Aedes aegypti,* the most important of the *Aedes* species, is considered highly anthropophilic, and breeds in man-made containers (Getachew *et al*., 2015) and is common in urban settings (Patrick *et al*., 2018). Improper disposal of waste containers provides perfect breeding environment for *Ae. aegypti* mosquitoes. For example, in coastal Tanzania, used tires and disposed containers were identified as commonest aquatic habitats for *Ae. aegypti* (Mathias *et al*., 2017; Mboera *et al*., 2016). However, less is known regarding the ecology of these vectors in inland Tanzania, including small towns, secondary cities and rural settings. This is important to understand distribution of the vectors across the country, but more importantly to prevent introduction or spread of *Aedes*-borne diseases. To ensure effective control, such ecological studies should be complemented with investigations on susceptibility to commonly-used public health insecticides (Chan, 2012; World Health Organization, 1997). This study was therefore conducted to investigate spatial distribution of *Ae. aegypti* in Ifakara town and surrounding wards in south-eastern Tanzania, characterize aquatic habitats of the mosquitoes in the area, and to assess susceptibility of the mosquitoes to insecticides commonly used for vector control.

1.3 Rationale of the problem

Assessing the distribution pattern and susceptibility profile of *Ae*. *Aegypti* in the area will allow us to further understand at fine scale, the areas and conditions favoring survival and proliferation of *Ae. aegypti* mosquitoes. As a result, the knowledge from this study will be useful when planning control measures before, during and future potential arboviral outbreak scenarios in the area and in similar settings.

1.4 Objectives

1.4.1 General objective

To investigate spatial and seasonal distribution of *Aedes aegypti* mosquitoes in Ifakara town and surrounding wards in south-eastern Tanzania, and assess its susceptibility to major insecticides.

1.4.2 Specific objectives

(i) To map the distribution and densities of adult *Ae. aegypti* in Ifakara town and surrounding wards.
(ii) To identify and characterize aquatic habitats of *Ae. aegypti* mosquitoes in the area.
(iii) To assess insecticide resistance status of *Ae. aegypti* mosquitoes against insecticides commonly used in public health.

1.5 Research questions

(i) What are the distribution patterns and densities of *Ae. aegypti* mosquito larvae and adults in Ifakara town and surrounding wards?
(ii) What is the susceptibility status of *Ae. aegypti* mosquitoes in Ifakara town and its surrounding wards?

1.6 Significance of the research study

The surveillance of this study will provide the baseline information regarding the presence of arboviral vector in rural and small growing town. It is also providing a basis evaluation of pathogen transmission and control options as well.

1.7 Delineation of the study

Previous studies on *Aedes*-borne diseases have been done in urban areas, but the ecology remains poorly understood in small towns and rural settings, thus the available information is limited. This dissertation is looking at the *Aedes* distribution in small town and susceptibility profile to common insecticides. Up to date this study stands as the available literature on the presence of this vector in small growing towns in Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Aedes aegypti* mosquitoes and the pathogens it transmits

Aedes aegypti is cosmopolitan mosquito specie. By physical appearance it has black body with white scales patterns (Fig. 1) (Higa, 2011). *Aedes aegypti* is an anthropophilic mosquitoes and most commonly found in and around people's dwellings (Dalgleish *et al*., 2007). Humans not only provide blood for its reproduction but also the conducive environment for its survival and development such as disposed containers for its breeding sites (Higa, 2011). Therefore, it's abundance and distribution are highly influenced by surrounding environment. This vector transmits arboviral infections such as Dengue fever, Yellow fever, Chikungunya and Zika (Amarasinghe *et al*., 2011; Gubler, 2004; Musso & Gubler, 2016; Patrick *et al*., 2018). These diseases are normally termed as *Aedes*-borne disease or arboviral diseases.

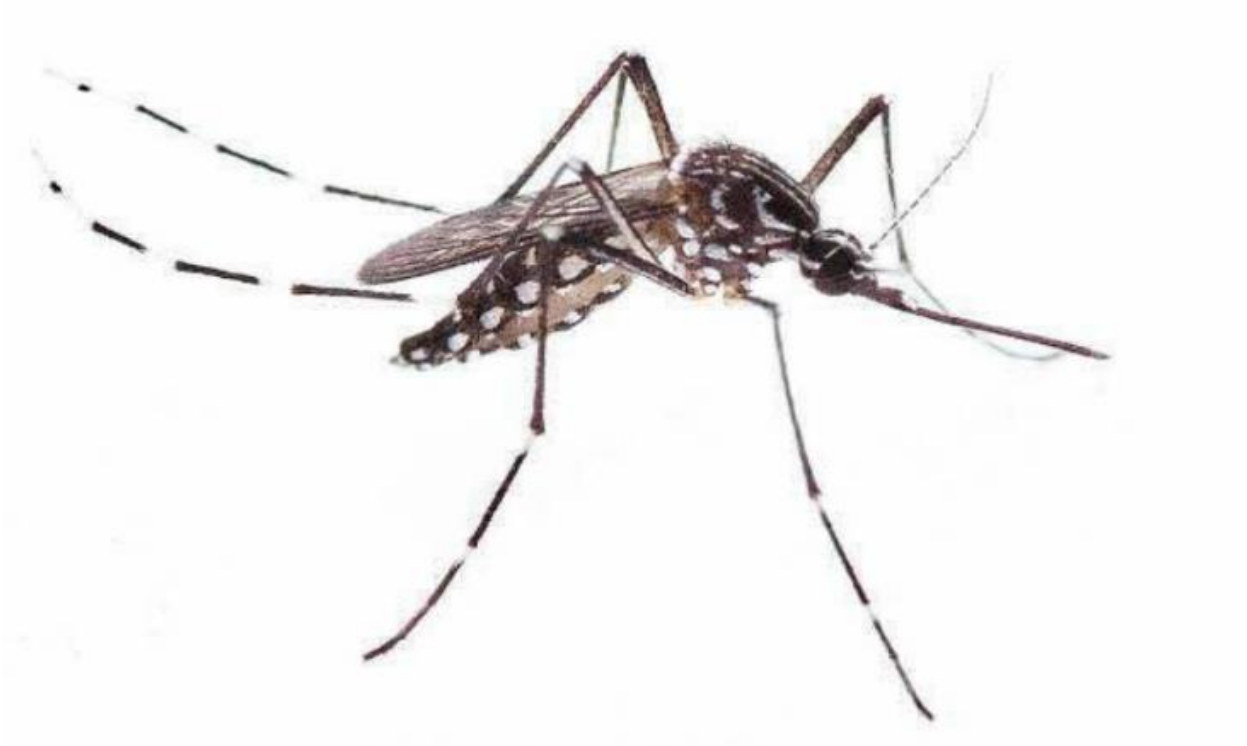

Figure 1: *Aedes aegypti* mosquito (Picture by PixelBase Ltd, Dar es Salaam)

2.2 Impacts of *Aedes*-borne disease

Diseases transmitted by *Aedes* are posing significant impact globally. It is estimated that more than 80% of the world's population is at risk of one vector-borne disease. Malaria and *Aedes*-borne infections being the most common (Golding *et al*., 2015). Africa continent is estimating 70% which is equivalent to 831 million people being at risk of getting at least one *Aedes*-borne infection (Weetman *et al*., 2018). The leading *Aedes*-borne disease is Dengue fever which constitutes about 750 million African people at risk followed by Chikungunya and Zika (Weetman *et al*., 2018). The burden for *Aedes*-borne infections are not certain because there still insufficient diagnostic tools in many localities (Jaenisch *et al*., 2014), resulting in most cases being misdiagnosed and therefore underreported (Petti *et al*., 2006). When *Aedes*-borne infection occurs in any locality are normally termed as dramatic outbreak. Dengue cases often spread fast, and there is no appropriate medication rather than treating the symptoms such as fever and pains. Moreover, these infections do not have vaccination except yellow fever (Garske *et al*., 2014) and now there is ongoing trials for developing dengue fever vaccines (Biswal *et al*., 2019).

2.3 *Aedes aegypti* aquatic habitat

Aedes mosquitoes do not need big water sources to lay eggs. Instead, small storage containers such as coconut husks, bottle rids and anything that can hold water for more than three days are enough for them to use as oviposition sites (Fig. 2). Thus, they are referred as container-breeding mosquitoes (Getachew *et al*., 2015). Moreover, *Aedes* eggs can withstands dryness hence making their populations resilient and more sustainable (Walker *et al*., 2011). They also have ability to lay eggs in tiny locations that cannot easily be found in habitat removal campaigns. For instance, in roof gutters and hidden small spaces in trees (Ngugi *et al*., 2017; Ritchie & Montgomery, 2002). When these locations are not directly exposed to the sun, they store water for periods long enough to breed mosquitoes. Other habitats common in housing surroundings include discarded used tires, tarps, buckets, flower pots, animal feeding containers, neglected tub, overwatering, pond etc. Presence and densities of these containers can influence the density of *Aedes aegypti* mosquitoes as they provide a conducive environment for breeding.

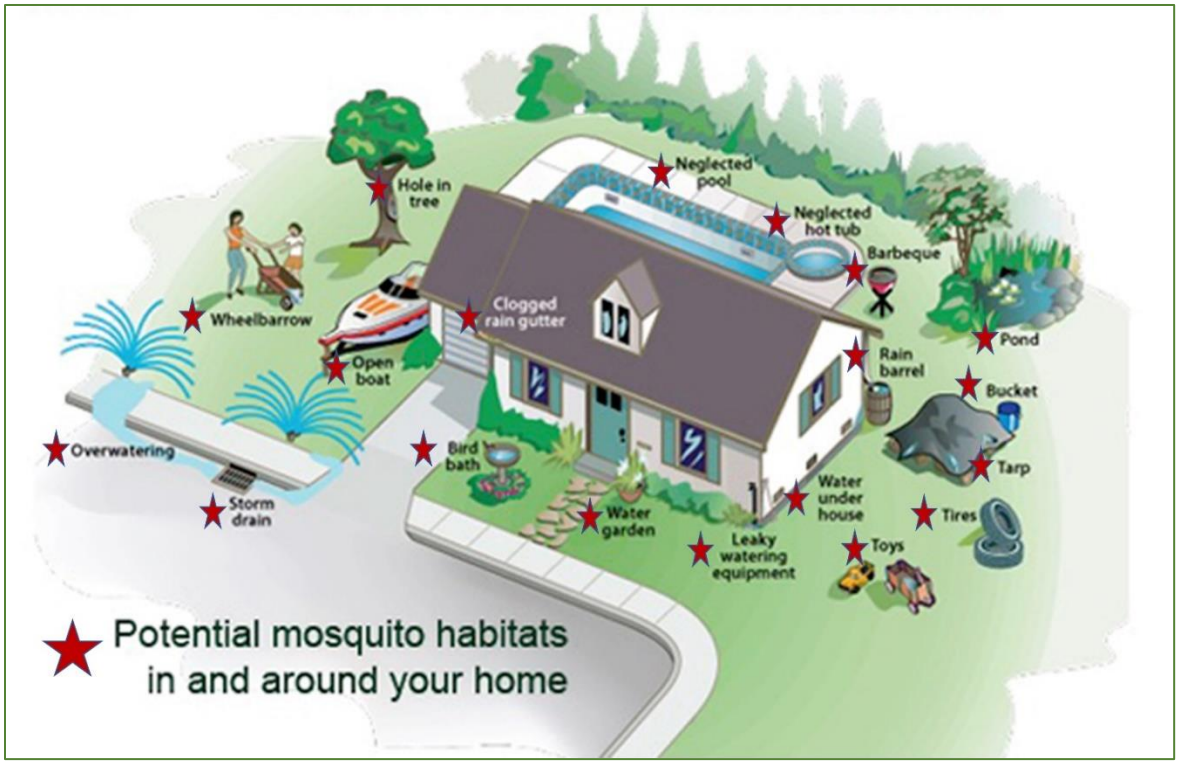

Figure 2: Potential breeding sites for *Aedes aegypti* around human house (Picture obtained from Miami's Community Newspapers)

2.4 Current approaches used for entomological surveillance

To be prepared in case of outbreaks, entomological surveillance is essential to understand mosquito behavior and geographical distribution. Larval surveillance is established by continuous search of immature *Aedes* in water holding objects available. Different indices are currently used to monitor areas with high risk for outbreaks. These include: (a) Container Index (proportion of containers infested with *Ae. aegypti* larvae or pupae), (b) House Index (proportion of houses infested with *Ae. aegypti* larvae or pupae), (c) Breteaux Index (number of infested containers per 100 houses) and (d) Pupae index (number of pupae obtained in 100 house) (World Health Organization, 1997, 2003b, 2016). When larval survey indicates low infestation ovitrap is normally used to target mosquito to lay eggs (World Health Organization, 1995).

Adult surveillance is mostly done by trapping mosquitoes and estimating the abundance base on the number of mosquitoes caught. The common traps used are: (a) BG sentinel trap, which uses artificial CO_2 sources or other synthetic lures (e.g. BG Lure) to lure mosquitoes (Ball & Ritchie,

2010); mosquitoes follow these cues upwind in anticipation of real humans (Kröckel *et al*., 2006), (b) Gravid *Aedes* traps, which operate like ovitraps but they only prevent mosquito from escaping by either funnel (Ritchie *et al*., 2014b) or glue, (c) Mechanical aspirators, used to collect male and female resting mosquitoes, to give a good representation of *Aedes* population since they capture all the bloodfed, gravid and unfed (Centers for Disease Control and Prevention, 2017), and (d) Human Landing or Animal biting counts, which are effective at quantifying human-biting mosquitoes, but are risky particularly when there is transmission of arbovirus in the area (Centers for Disease Control and Prevention, 2017).

2.5 Current approaches for the control of arboviral infections

Control of *Aedes*-borne infections is currently hampered by various factors, such as absence of proper medication and vaccines and lack of early detection of cases. Though a recent dengue fever vaccine has shown great progress in clinical trials (Biswal *et al*., 2019), there is still no vaccine widely available for different populations to prevent many of the *Aedes*-borne diseases. Therefore, the easiest and practical approach for preventing infections is to target the vector (Focks, 2003). Vector control management needs a clear understanding of the main breeding sites and factors associated with their density.

There are three main interventions currently recommended for *Aedes* control. First is environmental sanitation and management, permanent destruction of breeding sites. When sanitation facilities are improved such as reliable supply of piped water, trash removal programs and larvicide application to where habitats cannot be removed (Centers for Disease Control and Prevention, 2017). Second is spraying of insecticides to places where mosquitoes rest. This approach should always be accompanied with insecticide resistance monitoring (Mnzava & Pinzon, 2016). Third is personal protection, such as wearing long sleeves, socks, the use of topical and spatial repellents, the use of bednet and screening of the houses so as to reduce human vector interaction (Centers for Disease Control and Prevention, 2017).

2.6 Current approaches to assessment of insecticide resistance status of *Aedes spp.*

Assessment of insecticide resistance is important to determine how local mosquito populations respond to public health pesticides, but also to select the most effective insecticides to use. Today,

it is mainly done by two approaches. First, is CDC bottle bioassay whereby the bottle is coated with known concentration of insecticide and left to be observed for 2 h (Fig. 3a) (Brogdon & Chan, 2010). Second, is WHO bioassay whereby the tubes are inserted with treated paper with either discriminating or intensity known concentration and left to be observed for 1 h (Fig. 3b) (World Health Organization (WHO), 2016). These approaches have been developed to examine the resistance status of mosquitoes and respective dose of the insecticide which is effective at killing. Throughly understanding of the resistance profile is important especially to places where the chemical is mainly used for interventions in vector control so as to maintain efficient usage of chemicals.

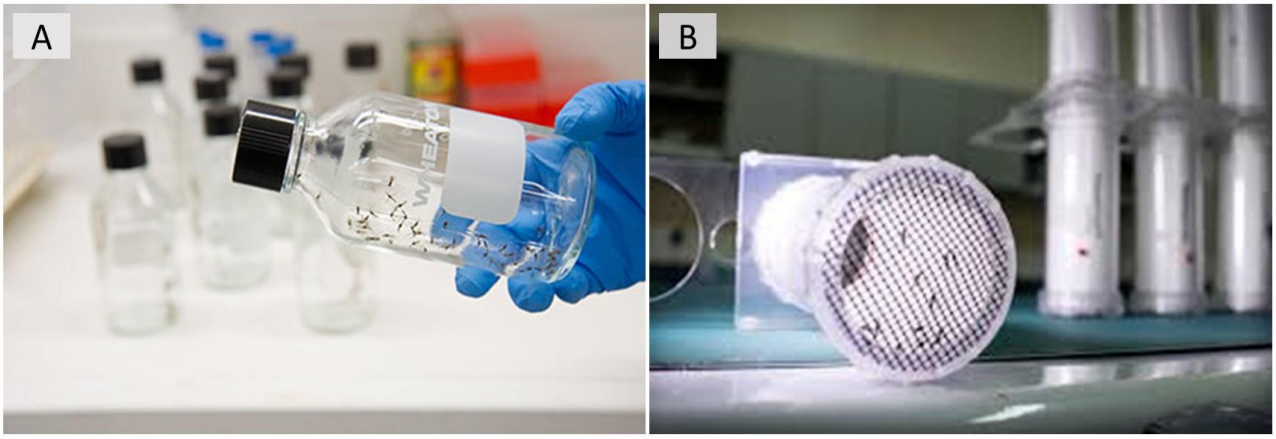

Figure 3: Insecticide resistance monitoring
Key: A = CDC bottle bioassay, B = WHO bioassay

This thesis provides details of a study conducted to investigate spatial and seasonal distribution of *Ae. aegypti* in Ifakara town and surrounding wards in south-eastern Tanzania. Ifakara is a fast-growing small town, where the risk of *Aedes*-borne infections is also on the rise. The study characterized aquatic habitats of the mosquitoes in the study area and also assessed susceptibility of the mosquitoes to insecticides commonly used for vector control.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Mosquito collections and surveys for *Aedes* immatures were conducted in Ifakara town and surrounding wards, namely, Lipangalala (-8.16428, 36.68964), Viwanja Sitini (-8.13512, 36.68413), Mlabani (-8.13952, 36.68964) and Katindiuka (-8.13154, 36.71165), all in the Kilombero river valley in south-eastern Tanzania (Fig. 1). The area has an average of 270 m altitude above the sea level. It is surrounded by Udzungwa mountain national park from northwest and Mahenge hill in the south. Average rainfall ranges from 1200 to 1800 mm per annum, relative humidity from 51% to 71% and temperatures from 20˚C to 32.6˚C (WorldData.info, 2019). The area experiences short rains in October and December, which is interrupted by dry months from January to March, after which heavier rains continue from April till May/June. Dry season is between July and September. Two of the wards, Ifakara town and Viwanja Sitini are characterized as urban, while the other three, Mlabani, Katindiuka and Lipangalala are rural. It is a rapidly growing area with total population now (2019) estimated at 67 500 people from country annual growth rate of 2.7%. Last census was conducted in 2012 Ifakara had a population of 55 956 people (National Bureaau of Statistics, 2013).

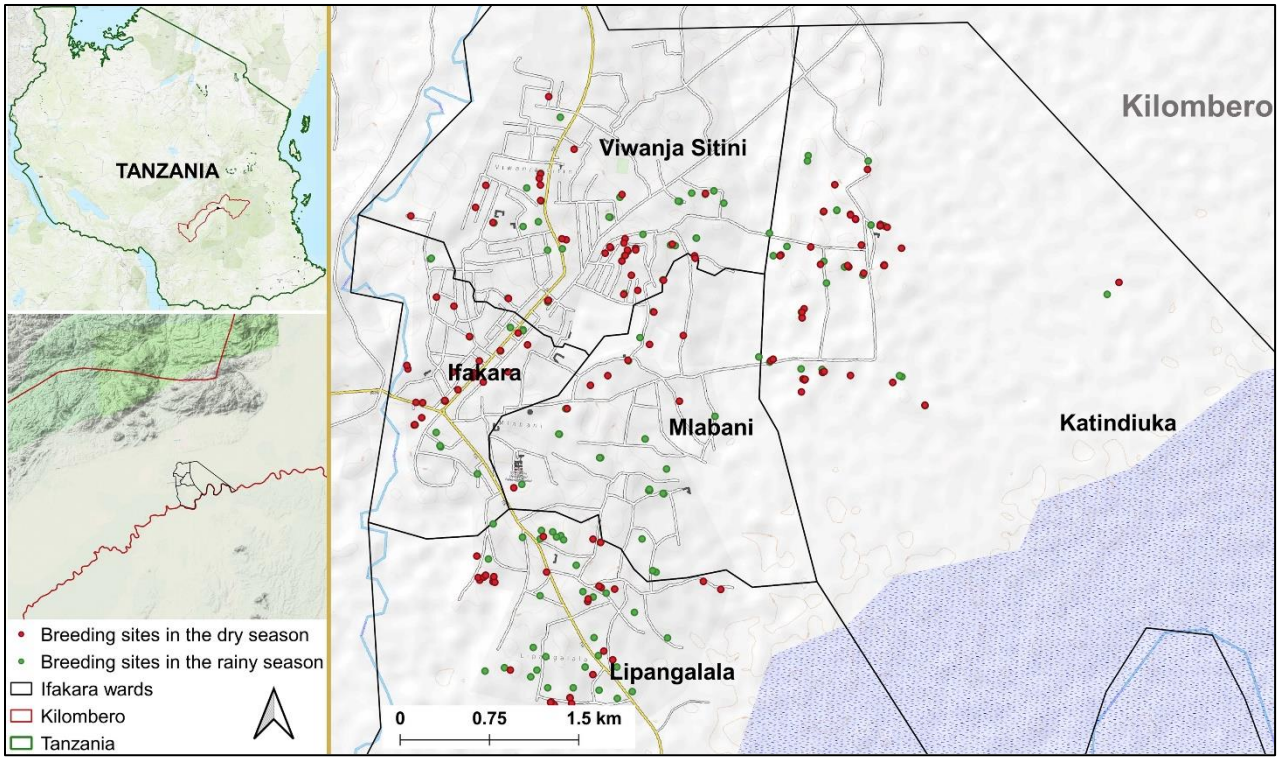

Figure 4: Study area: a map of Ifakara town and its surrounding wards showing locations where *Ae. aegypti* immatures were sampled

3.2 Sampling site selection

Initially the study area was divided into equal-sized grids of 200 m × 200 m using ArcGIS version 10.4 software, as previously used by Mwangungulu *et al*. (2016), and each grid assigned a unique identifier (Fig. 5). The grids were overlaid with household geo-location data initially collected by Ifakara Health Institute's Health and Demographic Surveillance System (Geubbels *et al*., 2015). The population data was then updated using population density maps from both Google satellite imagery and a high resolution settlement layer (HRSL) created by Facebook Connectivity Lab and Centre for International Earth Science Information Network (CIESIN) (Facebook Connectivity Lab & Center for International Earth Science Information Network, 2016).

From each ward, 34 grids containing human habitation and/or buildings were selected for mosquito sampling (both adult and larvae), thus creating a set of search grids. For each search grid, houses or buildings nearest to the centroid were identified as a starting point for the *Aedes* habitat searches. Whenever the centroid point had no building or building owner refused to consent, the nearest consenting household was selected for putting the trap. These became the starting points for larval

searches. From the starting point, we searched for all potential aquatic *Aedes* habitats within a 100 m radius, visiting each search grid twice in dry season and twice in rainy season. We also mapped important features such as schools, marketplaces, worship areas, health facilities and water pumps using handheld GPS receivers (Magelan eXplorist GC, USA).

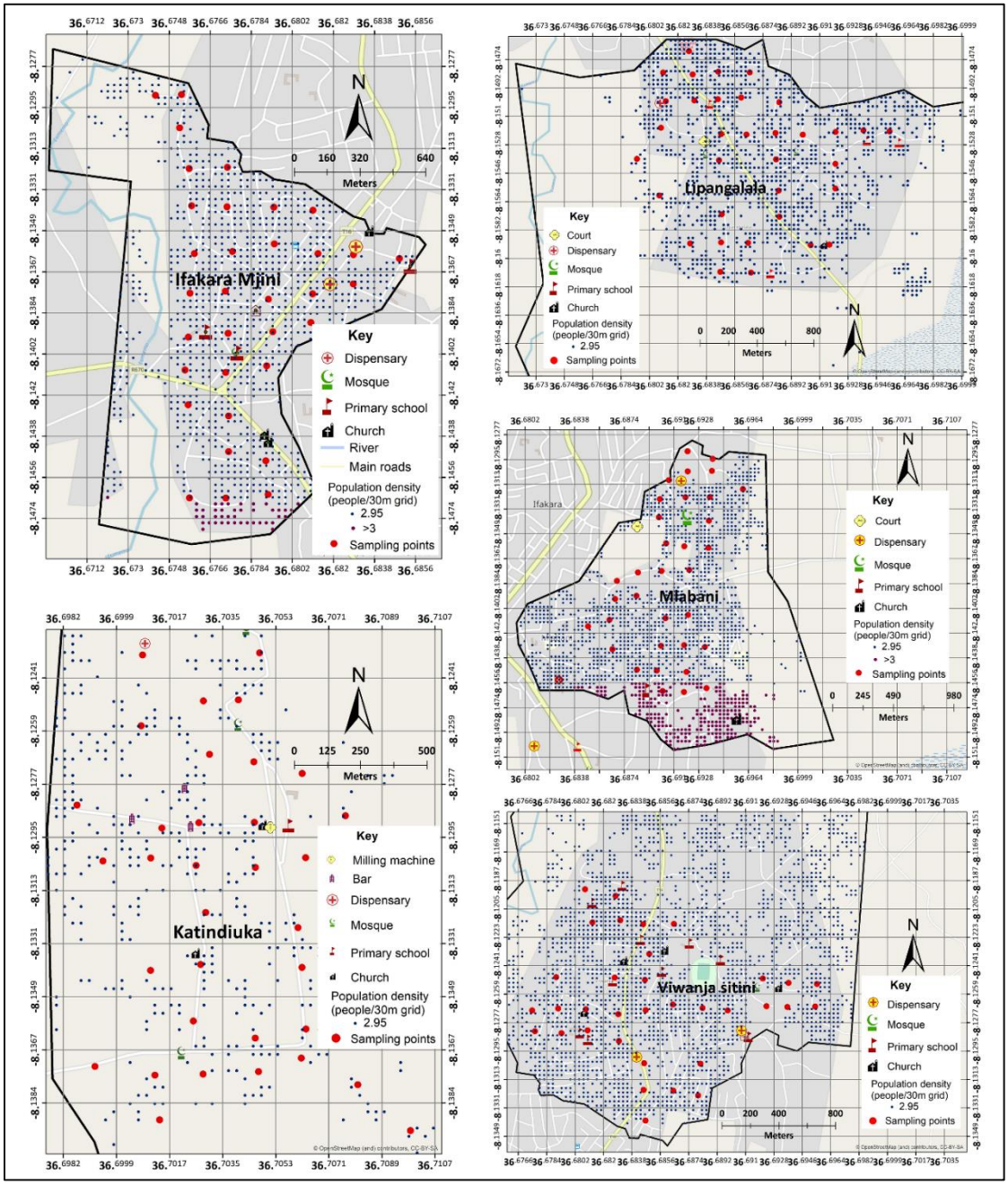

Figure 5: Selected grids in study area which were sampled for larval survey in both dry and rainy seasons

3.3 Sampling adult *Aedes* mosquitoes using Gravid *Aedes* trap (GAT)

In this study we used GAT traps to sample *Aedes* mosquitoes looking for breeding sites (Eiras *et al*., 2014; Ritchie *et al*., 2014a). Clean water and nonanal were added to the GAT traps as oviposition cues to attract mosquitoes. The nonanal used was replaced after every six months. In the trap black screen mesh were replaced with Pyrethroid-impregnated blue screen mesh to kill the trapped mosquitoes (Fig. 3). The GAT was positioned outside the house and was placed in shaded area where there is no direct wind, rain and sunlight.

A total of 20 GATs were used for mosquito collection in all study wards. For a complete round each ward needed four traps whereby, one was kept as sentinel (fixed to capture temporal variation) and other three were rotating in the other grids (for capturing spatial variation). The mosquitoes were retrieved in the morning from 0730 -1000 h after every four days. In the same period the traps were moved from one grid to another until all the grids had been sampled. This was to maintain the sampling process to be repeated in each grid. All retrieved mosquitoes were sorted into respective taxa using a morphological identification key (Huang & Ward, 1981). Mosquitoes were counted and recorded with the grid id, date, time and GPS location. The number of mosquitoes was used to estimate the abundance of mosquitoes.

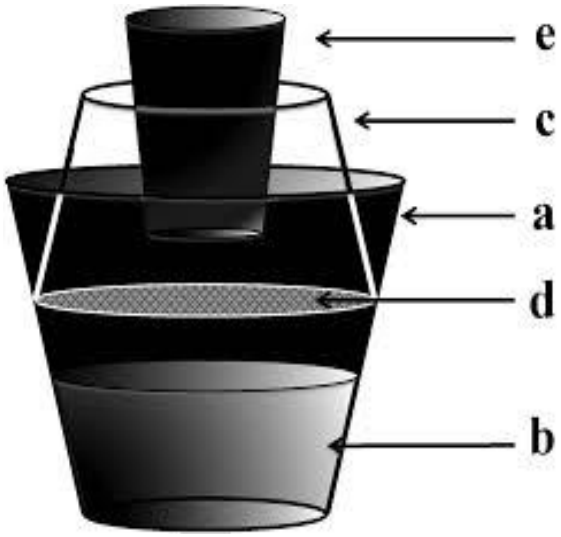

Figure 6: Basic components of GAT

Key: a = black matte bucket base, b = water; translucent chamber, d = black screen mesh and e = black funnel (Eiras *et al*., 2014)

3.4 Sampling of immature *Aedes* mosquitoes and habitat characterization

Sampling for *Aedes* mosquitoes and characterization of their habitats took place from November 2018 to February 2019 with a break of December (dry season) and from April to May 2019 (rainy season). The search for immatures focused on various natural and artificial water-holding objects such as tree holes, used tires, wells and discarded containers and animal feeding containers. Others included coconut shells, tarpaulins, broken glasses and other small objects that could potentially hold water longer than three days. All sites with *Ae. aegypti* larvae or pupae were geo-referenced using handheld GPS.

The habitats were then characterized by: (a) their location, (b) size, (c) apparent water color, (d) presence of vegetation, (e) presence of shading (f) source of water in the habitat, (g) whether the habitat was movable or not, and (h) environmental and social activities surrounding the habitats. We sampled larvae and pupae from each of the identified habitats using standard 350 ml dippers, or a smaller 70 ml dipper in cases where habitats were too small to use standard dipper. The larvae and pupae were placed in white trays for morphological identification using pictorial keys by US Center for Disease Control (Center for Disease Control, 2017). They were then sorted, counted and data recorded by habitat type, location and survey instance. All *Aedes* larvae were transferred to the vector biology laboratory (VectorSphere), at Ifakara Health Institute (IHI) for rearing.

3.5 Mosquito rearing and identification of emergent adults

At the VectorSphere, the larvae were placed in labeled basins and fed on Tetramin® baby fish food, and water changed every three days to facilitate larval growth. Rearing was done at temperatures of 26°C ± 2 °C and relative humidity of 82% ± 10%. Pupae were collected every morning, counted and transferred to netting cages measuring 30 cm × 30 cm × 30 cm. Emergent adults were fed on 10% glucose, and morphologically identified under stereo a microscope using the morphological keys (Centers for Disease Control and Prevention, 2017; Huang & Ward, 1981).

3.6 Bioassays for insecticide susceptibility

Bioassays were performed following WHO Insecticide Susceptibility Test Guidelines (Mnzava & Pinzon, 2016; World Health Organization (WHO), 2016). Female *Ae. aegypti* mosquitoes, 3-5

days old from each ward were tested against commonly used insecticides, ensuring to cover all major insecticide classes as follows: two pyrethroids both type I and II (deltamethrin; 0.05% and permethrin; 0.75%), one organochloride (dieldrin; 4%), one organophosphate (pirimiphos-methyl; 0.25%) and one carbamate (bendiocarb; 0.1%). In each experiment 120-150 mosquitoes were used and equally divided into six WHO holding tubes, so that each tube had 20-25 mosquitoes per test. They were then transferred into test tubes treated with a different chemical. Another two tubes were left as controls and the mosquitoes observed for 60 min. The number of mosquitoes knocked down was recorded at 10, 15, 20, 30, 40, 50 and 60 min. after which the mosquitoes were transferred back into holding tubes and provided with 10% glucose solution, under 28.0 °C ± 1.0 °C and 80% ± 10% relative humidity. Mortality was observed after 24 h in all treatment tubes and controls.

3.7 Measurements of mosquito wing lengths

Mosquitoes from different wards were assessed their wing length. Reared mosquitoes were anaesthetized in refrigerator at -10°C. One wing per mosquito was chopped from both male and female mosquitoes and placed on glass slide. Drops of distilled water were used to fix the wings onto the slides. Wing lengths were then measured, as distance from the apical notch to the auxiliary margin of each wing, under stereo zoom microscope using a micrometer ruler.

3.8 Data analysis

First, descriptive analysis was done to compare larval densities in different wards and seasons. Densities obtained from the 70 ml dipper were correlated to those from the standard 350 ml dipper and a correlation coefficient calculated across all collections. Using this coefficient, the densities by small dipper were all converted into standard dipper, so that all subsequent analysis was done based on the standard dipper. Second, Generalized Linear Models (Anjali *et al*., 2019) following a Poisson distribution for count data were used to model the number of larvae collected per dipper as response variable against season and habitat type as fixed factors. Logistic regression was also used to assess the association between the presence of *Ae. aegypti* within different habitats characteristic. The relative risk (RR), odds ratios (OR) and their 95% CI were estimated and

reported. Third, *dabestr* package was used to display mean differences of mosquito abundance (adult and larvae) between wards and seasons.

Larval indices, namely Container Index (proportion of containers infested with *Ae. aegypti* larvae or pupae), House Index (proportion of houses infested with *Ae. aegypti* larvae or pupae) and Breteaux Index (number of infested containers per 100 houses) were also calculated by ward and season (World Health Organization, 2017; World Health Organization, 1997). Mosquito wing lengths were compared using one-way ANOVA, followed by Tukey's *post-hoc* tests to assess mean differences between-ward for both male and female mosquitoes. Susceptibility status of *Ae. aegypti* was computed according to WHO guidelines (World Health Organization (WHO), 2016), log-probit analysis was used to compute mean duration at which 50% (KD_{50}) and 95% (KD_{95}) of the exposed mosquitoes were knocked down. The statistical analyses above were done using the open-source R statistical software, version 3.231 (R Development Core Team, 2016).

Spatial and seasonal distribution were analyzed by geostatistical in ArcGIS Version 10.4 (ESRI, USA). Inverse Distance Weighted (IDW) interpolation technique (Bailey & Gatrell, 1995; Santoso *et al*., 2018) was used to visualize the hotspots of the mosquito densities (adult and larvae). Representation of IDW maps show the patterns based on the distance from one observed point to another. Known values (number of larvae) were used as key input feature to estimate unknown locations within 400 m range. Distance used in analysis was selected according to the average flight range of *Aedes* mosquitoes (Verdonschot & Besse-Lototskaya, 2014). Geo-processing extents and masks were defined to match the study area.

3.9 Ethics statement

Approval for conducting this study was obtained from institutional review board of Ifakara Health Institute (Ref: IHI/IRB/No: 07 – 2019), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR), (Ref: NIMR/HQ/R.8a/Vol. IX/2555). Meeting with local leaders following an information session to highlight objectives, benefits and risks associated with the study, informed consent was obtained from all owners of all houses or buildings around which the mosquito surveys were conducted.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Adult mosquito trapping

A total of 926 mosquitoes were collected from November 2018 to June 2019. Of all collected mosquitoes 431 (46.5%) were identified as *Aedes aegypti*, 487 (52.5%) as *Culex*, 8 (0.01%) as other *Aedes* and 13 (0.01%) as *Anopheles* mosquitoes. In both seasons Katindiuka trapped many mosquitoes compared to other wards followed by Ifakara town. While Lipangalala was consistently yielding few mosquitoes (Table 1).

Table 1: Descriptive summary of adult mosquitoes caught in each study ward per season

Ward	Dry season				Rainy season			
	Aedes aegypti	*Culex*	Other *Aedes*	Total	*Aedes aegypti*	*Culex*	Other *Aedes*	Total
Ifakara town	39	59	5	103	67	35	0	102
Katindiuka	101	94	1	196	74	64	1	139
Viwanja sitini	22	40	0	62	21	28	0	49
Mlabani	6	58	0	64	52	32	1	85
Lipangalala	19	44	0	63	30	33	0	63

4.1.2 Larval indices

A total of 1515 breeding sites were visited in the dry season and 1933 in rainy season. Of these, 286 (18.87%) in dry season and 283 (14.64%) in rainy season were positive with *Aedes* immatures. The proportion of infestation varied across wards and seasons as summarized in Table 2. In the dry season, high Container Indices (CI) were observed in Katindiuka, Viwanja Sitini and Ifakara Town wards, while in rainy season, high CIs were in Ifakara town, Viwanja sitini and Lipangalala wards.

With regard to House Indices (HI), 2315 and 2832 houses were visited in dry and rainy season surveys, of which, 114 (4.9%) and 186 (6.6%) had at least one positive breeding site respectively.

Lipangalala ward had the highest HI during the dry season, while Ifakara town had highest HI in rainy season. Compared to dry season, HI increased during rainy season in all wards expect Lipangalala (Table 2). It was also observed that Viwanja Sitini ward had highest Breteaux Index (BI) in both the dry and rainy season.

Table 2: Summary of *Ae. aegypti* larval survey indices by ward and seasons

Wards	Dry season			Rainy season		
	CI (%)	HI (%)	BI (%)	CI (%)	HI (%)	BI (%)
Ifakara town	21.4	4.18	16.74	27.4	7.12	9.54
Katindiuka	18.7	4.45	9.37	11.2	6.78	7.22
Viwanja sitini	29.5	6.67	20.28	26.2	6.75	15.25
Mlabani	13.01	3.33	5.12	11.9	6.58	10.53
Lipangalala	21.4	6.44	8.44	19.6	5.11	11.11

Key: CI = Container Index: ratio of larval infested to total inspected containers, HI = House Index: ratio of larval infested to all inspected houses and BI = Breteaux Index: ratio of positive containers per 100 houses inspected

4.1.3 Densities of *Ae. aegypti* immatures and their aquatic habitats

A total of 63 470 larvae or pupae were collected from all wards. Of these, 76.3% (n=48 459) were *Ae. aegypti*, 20.9% (n=13 253) were *Culex* and 2.8% (n=1758) were identified as other *Aedes* species mosquitoes. In the dry season surveys, Ifakara town produced nearly one third of all immature *Aedes* and more than one third of immature *Culex*. In the rainy season however, Viwanja Sitini had more than one third of *Aedes* immatures, while Katindiuka had more than half of *Culex*. Most *Culex* were found in the dry season, while *Aedes* were more prevalent in the rainy season (Table 3).

Overall, most *Aedes* larvae were from used tires and clay pots followed by other containers such as discarded tins, buckets, drums and animal feeding pots (Fig. 7). However, coconut tree holes and flower pots had far higher numbers of larvae per dip compared to all other habitat types, in the dry season (Table 3). The likelihood of getting larvae in individual tree holes was three times higher than in used tires (RR=3.00 [2.58-3.50], P<0.01). However, in the rainy season, higher larval densities were observed in other habitats (Table 4).

Table 3: Sampled populations of *Aedes* and *Culex* larvae collected in all aquatic habitats

Wards	Dry season				Rainy season				Total	
	Aedes		*Culex*		*Aedes*		*Culex* larvae		*Aedes*	*Culex*
	N	%	N	%	N	%	N	%	N	N
Ifakara town	5325	32	4217	39	6769	20	0	0	12094	4217
Katindiuka	2845	17	1240	11	2383	7	919	37	5228	2159
Viwanja sitini	3527	21	3116	29	11652	35	0	0	15179	3116
Mlabani	1833	11	826	8	7698	23	15	1	9531	841
Lipangalala	3284	20	1386	13	4901	15	1534	62	8185	2920

Key: N = number of larvae collected and % = percentage of larvae by ward

Table 4: Larval densities in different aquatic habitats of *Ae. aegypti* mosquitoes in the dry and rainy seasons in the study area

Habitat type	Larvae (N)	Habitats (n)	Mean (95% CI)	RR (95% CI)	P- Value
			Dry season		
Used tire	844	51	16.5 (15.46-17.70)	1	
Clay pot	652	44	14.8 (13.7-16)	0.89 (0.80-0.99)	0.034
Container	93	24	3.9 (3.16-4.75)	0.23 (0.19-0.29)	<0.01
Flower pot	163	9	18.1 (15.53-21.12)	1.09 (0.93-1.29)	0.292
Pit	96	7	13.7 (11.23-16.75)	0.83 (0.67-1.02)	0.081
Tree hole	199	4	49.8 (43.3-57.17)	3 (2.58-3.50)	<0.01
Others	12	6	2 (1.14-3.52)	0.12 (0.07-0.21)	<0.01
			Rainy season		
Used tire	1276	55	23.2 (21.96-24.51)	1	
Clay pot	978	55	17.8 (16.7-18.93)	0.77 (0.70-0.83)	<0.01
Container	504	27	18.7 (17.11-20.37)	0.80 (0.72-0.89)	<0.01
Flower pot	273	17	16.1 (14.26-18.01)	0.69 (0.61-0.79)	<0.01
Pit	133	7	19 (16.03-22.52)	0.82 (0.69-0.98)	0.028
Tree hole	68	4	17 (13.4-21.56)	0.73 (0.57-0.94)	0.012
Others	119	5	23.8 (19.87-28.48)	1.03 (0.85-1.24)	0.79

Key: RR = risk ratio, CI = confidence interval, N = total number of larvae/dips and n = number of habitats

Category used as reference R = 1, means reported here are predicted from generalized linear model which is average of larvae per dipper to number of breeding sites. Used tire was selected as reference because they were present in all study sites. "*Others*" included positive breeding sites such as disposed shoes, coconut shells, tarpaulins, broken glasses and open plastic bottles.

Figure 7: Various breeding sites identified in the study area

Key: A = used tires used as seats, B = used tires kept for protecting trees from pests, C = disposed coconut shells, D = flower pots, E = animal feeding container, F = broken grasses, G = disposed containers, H = coconut tree holes, I = clay pots, J = small container and J = pit

4.1.4 Positivity of different habitat types for *Aedes* immatures

Positivity of the habitats for *Aedes* are summarized in Table 5. By assessing proportions for each type of habitat, it was determined that used tires were the most commonly infested with *Ae. aegypti* (89% positivity), followed by containers (86% positivity) and clay pots (82% positivity), garage

pits (64% positivity) and others (90% positivity). Majority of the positive breeding sites were movable, associated with human activities, or were found in and around residential areas, commercial places and garages. However, no clear statistical differences in likelihood of positivity for *Aedes*, across different habitat types or different sizes of habitats. We, however observed significantly higher *Aedes* positivity in rainy season than dry season. Also, number of positive habitats were higher if they had clear water than turbid water.

Table 5: Results of the logistic regression analysis showing positivity and negativity of habitats of different characteristics for immature *Ae. aegypti* mosquitoes

Parameter	Categories	N (%)			Univariate		Multivariate	
		Positive	Negative	Total	OR (95%CI)	P-Value	OR (95%CI)	P-Value
Habitat type	Used tires	89 (84)	17 (16)	106	1		1	
	Clay pot	81 (82)	18 (18)	99	0.86 (0.42-1.78)	0.68	0.55 (0.21-1.42)	0.216
	Container	44 (86)	7 (14)	51	1.2 (0.46-3.11)	0.70	1.07 (0.33-3.48)	0.904
	Flowerpot	22 (85)	4 (15)	26	1.05 (0.32-3.44)	0.93	0.62 (0.13-2.95)	0.551
	Pits	9 (64)	5 (36)	14	0.34 (0.10-1.15)	0.08	0.11 (0.01-2.54)	0.172
	Tree hole	7 (88)	1 (12)	8	1.34 (0.15-11.58)	0.79	0.92 (0.03-31.86)	0.962
	Others	10 (90)	1 (9)	11	1.91 (0.23-15.91)	0.55	2.98 (0.26-34.82)	0.383
Size	Large	36 (71)	15 (29)	51	1		1	
	Medium	129 (84)	25 (16)	154	2.15 (1.03-4.5)	0.042	1.73 (0.71-4.18)	0.2165
	Small	97 (88)	13 (12)	110	3.10 (1.35-7.17)	<0.001	0.98 (0.33-2.89)	0.966
Season	Dry season	97 (67)	48 (33)	145	1		1	
	Rainy season	165 (97)	5 (3)	170	16.3 (6.3-42.4)	<0.001	19.73 (6.61-58.94)	<0.001
Movability	Immovable	20 (74)	7 (26)	27	1		1	
	Movable	242 (84)	46 (16)	288	1.8 (0.74-4.6)	0.192	0.36 (0.03-5.24)	0.46

Parameter	Categories	N (%)			Univariate		Multivariate	
		Positive	Negative	Total	OR (95%CI)	P-Value	OR (95%CI)	P-Value
Turbidity	Clear	145 (88)	20 (12)	165	1		1	
	Turbid	109 (83)	22 (17)	131	0.68 (0.36-1.32)	0.254	0.79 (0.38-1.67)	0.5417
	Very turbid	8 (42)	11 (58)	19	0.10 (0.03-0.27)	<0.001	0.13 (0.04-0.44)	<0.001
Shades	Full	115 (86)	19 (14)	134	1		1	
	Partial	126 (81)	29 (19)	155	0.72 (0.38-1.35)	0.303	0.74 (0.35-1.60)	0.45
	None	21 (81)	5 (19)	26	0.7 (0.23-2.06)	0.511	0.43 (0.09-1.92)	0.27
Water source	Domestic	12 (63)	7 (36)	19	1			
	Rainwater	250 (84)	46 (16)	296	3.17 (1.19-8.45)	0.02	1.11 (0.28-4.39)	0.87

Key: OR = odds ratio, CI = confidence interval, N = number of breeding sites and Category used as reference R = 1

4.1.5 Spatial and seasonal distribution of adult *Aedes* mosquiotes

The distribution of mosquitoes varied between administrative wards. In both seasons, very high abundance of mosquiotes were marked in Katindiuka (Fig. 8) compared to other wards. The "hotspot" observed in the dry season were similar to rainy season but extended.

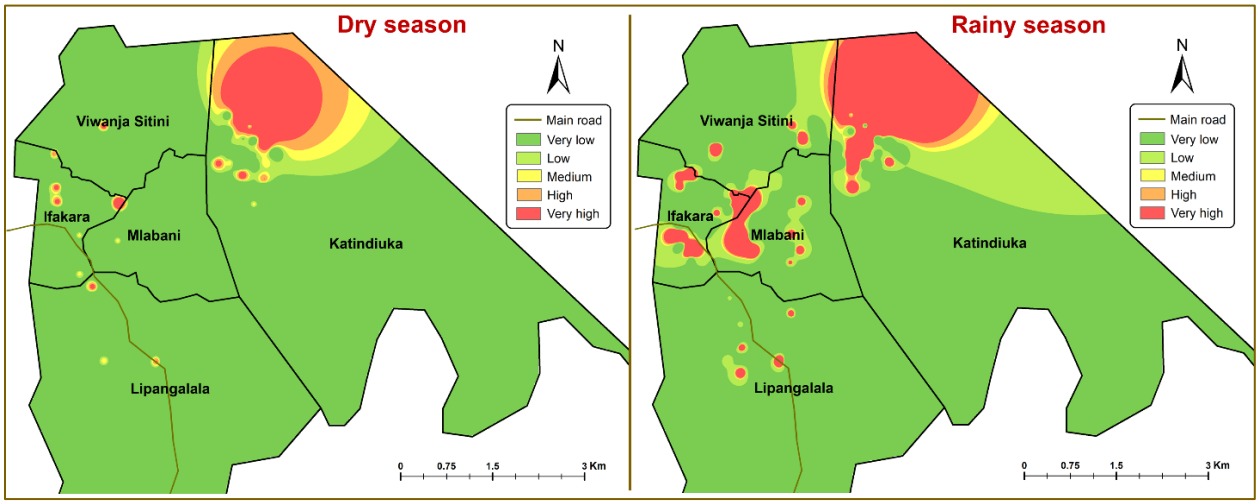

Figure 8: Spatial and seasonal distribution of *Aedes* mosquiotoes

Key: Very Low < 2 mosquitoes, Low = 3-4 mosquitoes, Medium = 5-6 mosquitoes, High = 7-8 mosquitoes, Very High > 9 mosquitoes

4.1.6 Spatial and seasonal distribution of *Aedes* immatures

The spatial distribution of *Aedes* immatures varied between dry and rainy season (Fig. 9). In dry season, the highest infestation was from the center of Ifakara town toward western parts of Katindiuka ward. In the rainy season on the other hand, most infested locations were in southern Lipangalala and in Viwanja sitini (Fig. 9).

Generally, fewer breeding sites were observed in dry season compared to rainy season in all study sites, though actual abundance varied significantly between sites. Ifakara town consistently had higher mean number of larvae than the other wards across seasons (Fig. 10). We also estimated the residual mean differences of larval abundance between study ward.

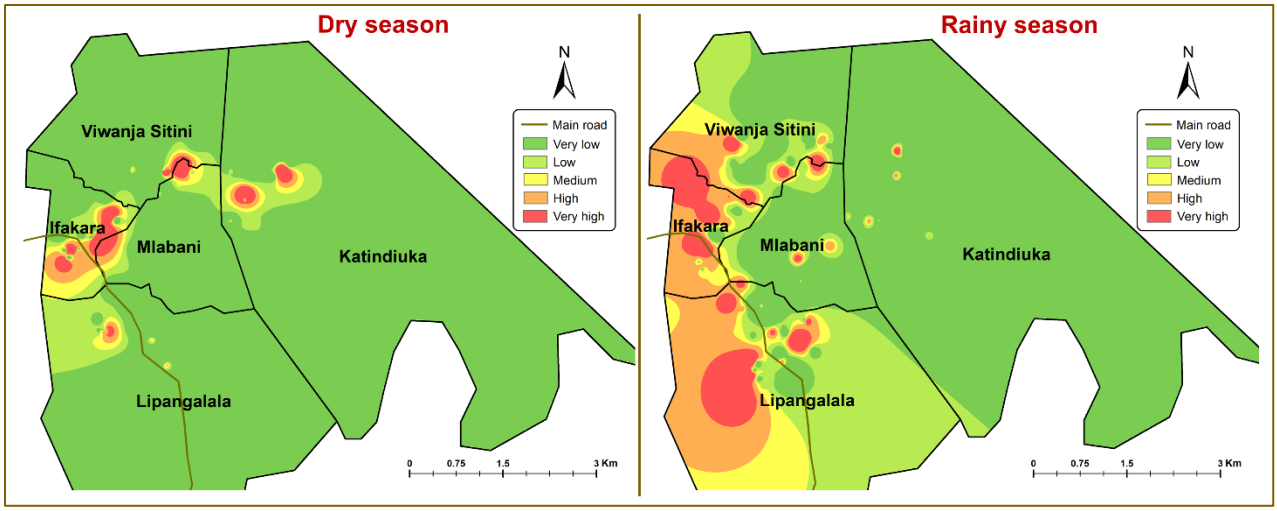

Figure 9: Spatial and seasonal distribution of *Aedes* larvae infested locations

Key: Very Low = 0-16 *Aedes* larvae/dip, Low = 17-20 larvae/dip, Medium = 21-23 larvae/dip, High = 24-28 larvae/dip, Very High = 29-37 larvae/dip

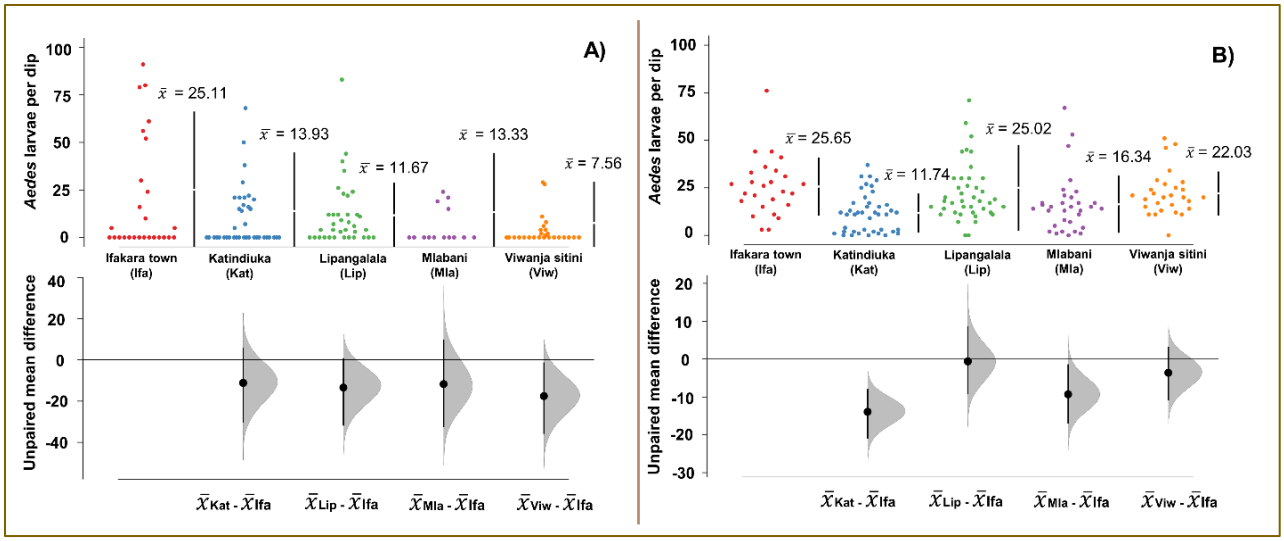

Figure 10: Estimated means of *Aedes* larvae/dip in Ifakara town and surrounding wards

Key: A = Dry season and B = Rainy season.

Estimation plots are used to portray the distribution of residual mean differences of larval abundance between study wards. The vertical lines represent mean ± confidence levels (the gap in the line is the mean). The filled curves indicate the resampled mean difference distribution of the larval abundances with reference to Ifakara town. Black vertical line indicates 95% confidence

level. Black dot indicates mean difference to the reference group. The significance is considered depending on how far the means of residual deviated from the refence line.

4.1.7 Susceptibility of *Aedes aegypti* mosquitoes to public health insecticides

Aedes aegypti females were generally susceptible to all four classes of insecticides. Only in few instances did *Ae. aegypti* show reduced susceptibility to carbamates, and pyrethroids. Confirmed resistance was detected against only bendiocarb in the rainy season tests (Fig. 11).

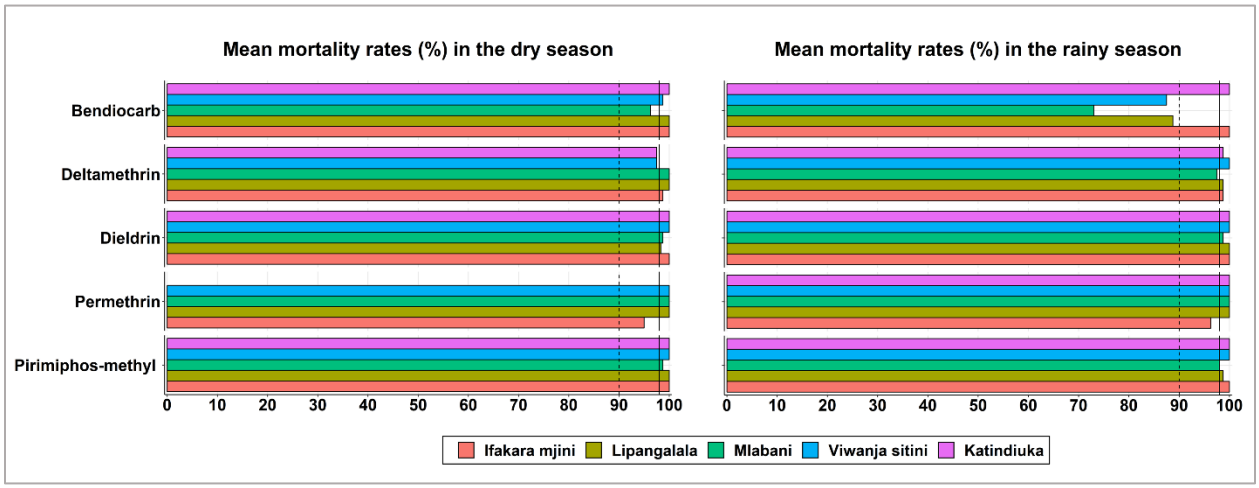

Figure 11: Mean mortalities demonstrating susceptibility status of *Ae. aegypti*

The solid lines (≥98% mortality) indicate that mosquitoes are fully susceptible to insecticide, while the dotted lines (90-98% mortality) indicate possible resistance requiring confirmation. Overall knockdown KDT_{50} and KDT_{95} ranged from 7 to 112 min and 13 to 159 min. respectively (Table 6). The knock down analysis revealed spatial and seasonal variation. Dieldrin and pirimiphos-methyl consistently achieved slower knock-down across wards, while bendiocarb and deltamethrin had quick knock-down. Knock-down times were not predictive of overall 24 h mortality. Often, mosquitoes were not affected by the insecticides during first 60 mins but mortality after 24 h was still high**.**

Table 6: Knock-down times of *Ae. aegypti* mosquitoes collected from different sites

Insecticide	Ward	Dry season		Rain season	
		KDT_{50}±SE (min)	KDT_{95}±SE (min)	KDT_{50}±SE (min)	KDT_{95}±SE (min)
Bendiocarb	Ifakara town	21.44 ± 4.52	28.68 ± 8.95	14.58 ± 6.28	30.26 ± 12.90
	Katindiuka	16.89 ± 3.05	22.15 ± 6.17	22.85 ± 6.68	39.34 ± 13.45
	Lipangalala	30 ± 5.82	41.16 ± 10.29	32.94 ± 8.04	53.86 ± 15.43
	Mlabani	25.13 ± 6.94	42.28 ± 13.66	30.77 ± 6.48	44.18 ± 11.70
	Viwanja sitini	28.91 ± 5.67	38.99 ± 10.08	39.77 ± 9.18	63.91 ± 18.76
Deltamethrin	Ifakara town	9.67 ± 3.56	14.11 ± 5.78	6.19 ± 5.9	17.16 ± 8.83
	Katindiuka	11.45 ± 4.42	19.95 ± 7.65	12 ± 9.60	37.44 ± 18.07
	Lipangalala	29.09 ± 46.16	31.59 ± 76.50	12.46 ± 3.44	18.52 ± 6.27
	Mlabani	7.2 ± 4.58	12.30 ± 5.27	16.41 ± 13.99	59.19 ± 30.42
	Viwanja sitini	7.12 ± 4.48	13.08 ± 5.75	17.64 ± 5.45	30.20 ± 11.55
Dieldrin	Ifakara town	36.02 ± 7.32	52.69 ± 13.26	75.43 ± 49.31	101.68 ± 103.19
	Katindiuka	40.73 ± 7.56	57.80 ± 13.96	22.9 ± 8.44	47.57 ± 17.40
	Lipangalala	43.32 ± 5.95	53.86 ± 10.68	85.57 ± 70.37	146.57 ± 154.15
	Mlabani	70.9 ± 33.93	102.46 ± 75.05	40.21 ± 8.70	62.23 ± 17.21
	Viwanja sitini	49.01 ± 6.89	62.59 ± 13.51	66.17 ± 370.887	70.70 ± 620.79
Permethrin	Ifakara town	12.69 ± 7.55	32.13 ± 14.93	7.2 ± 7.59	23.42 ± 12.58
	Katindiuka	-	-	10.56 ± 8.14	30.66 ± 15.25
	Lipangalala	8.52 ± 4.38	14.87 ± 5.95	12.28 ± 2.60	16.27 ± 4.68
	Mlabani	29.83 ± 7.21	47.19 ± 13.58	9.54 ± 8.73	30.60 ± 15.78
	Viwanja sitini	15.38 ± 4.41	24.73 ± 9.57	18.28 ± 3.17	23.45 ± 7.01
Pirimiphos-methyl	Ifakara town	75.66 ± 44.78	109.97 ± 95.41	71.03 ± 37.01	114.39 ± 83.36
	Katindiuka	78.03 ± 50.32	125.04 ± 109	26.66 ± 7.90	48.30 ± 15.75
	Lipangalala	79.14 ± 52.26	123.36 ± 111.14	32.36 ± 10.12	63.19 ± 22.59
	Mlabani	60 ± 15.83	84.06 ± 38.22	43.72 ± 8.41	63.61 ± 16.69
	Viwanja sitini	83.29 ± 102.4	108.97 ± 193.88	39.75 ± 14.95	84.60 ± 41.95

Key: N number of tested mosquitoes, SE standard error, KDT_{50} time taken for 50% of the tested mosquitoes to be knock-down, KDT_{95}% time taken for 95% of the tested mosquitoes to be knock-down. In each experiment there were six replicates and 120-150 *Aedes* female mosquitoes

4.1.8 Wing lengths of adult *Aedes aegypti* mosquitoes

Wing lengths, used here as a proxy for adult sizes of male and female *Ae. aegypti* ranged from 1.9 mm to 3.5 mm (Fig. 8). The mean wing sizes were 2.48 (±0.15) for mosquitoes from Ifakara town, 2.68 (±0.23) in Katindiuka, 2.73 (±0.20) in Lipangalala, 2.33 (±0.18) in Mlabani and 2.68 (±0.13) in Viwanja sitini. There was a significant difference in female mosquito's wing sizes across wards (ANOVA: F-statistic: 45.5 df =4, p<0.001). *Post hoc* analysis also revealed differences between pairs of wards (Fig. 8). Also, the mean wing length of female *Ae. aegypti* were generally larger than those of male *Ae. aegypti* (ANOVA: F-statistic: 365.9 df =1, p<0.001).

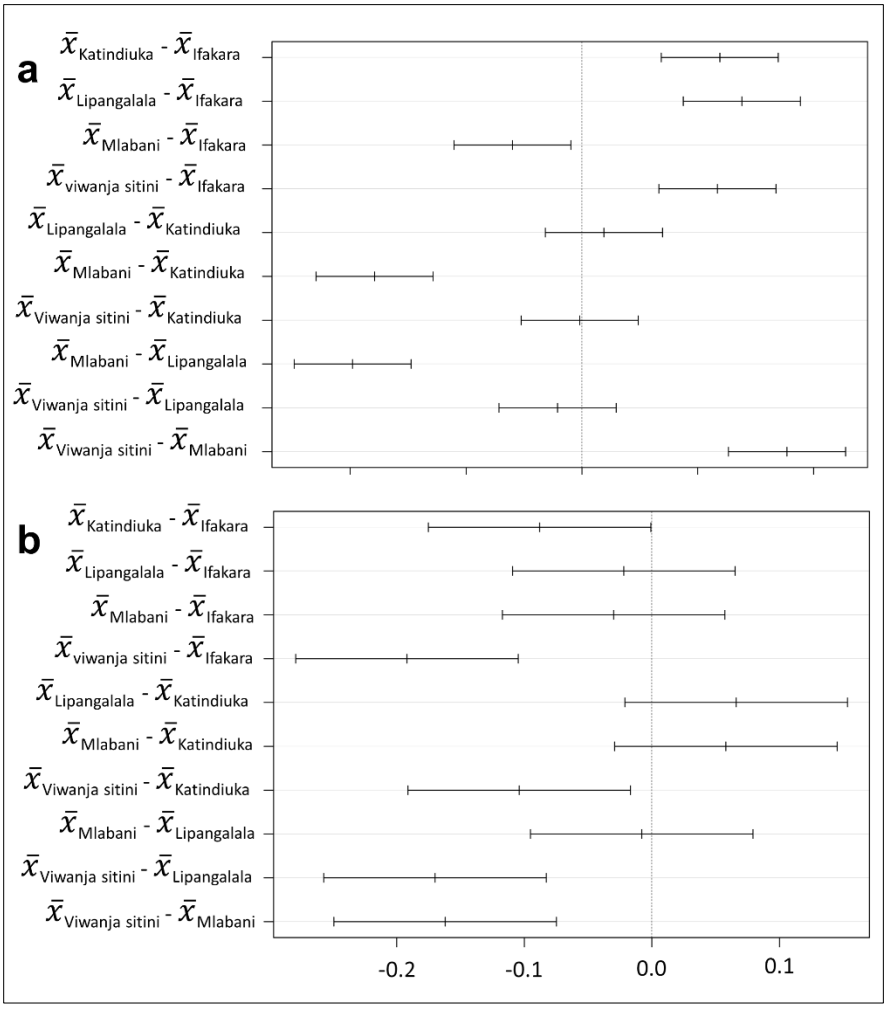

Figure 12: Differences in mean wing lengths between wards

Key: a = Female and b = Male

4.2 Discussion

In Tanzania, the majority of studies carried out to understand the ecology of arbovirus vectors are in response to outbreaks, and are often concentrated in large urban areas (Mboera *et al*., 2016). Basic ecological studies to understand the distribution and behaviors of the vectors, as well as their responses to interventions remain very few. This current study involved an exploratory survey of *Ae. aegypti* mosquitoes in a small town and its surrounding wards in south-eastern Tanzania. It therefore provides essential data on *Aedes* mosquitoes in this area where no outbreak has previously been reported, yet the risk is high. Given that there have been reports of arboviral infections such as Dengue and Chikungunya in neighboring districts (Chipwaza *et al*., 2014), it is crucial to invest in studies to improve our understanding of the ecology of the vectors, so as to improve control. This study therefore assessed three important aspects, namely: (a) spatial distribution of *Ae. aegypti* mosquitoes in Ifakara town and its surrounding wards in south-eastern Tanzania, (b) characteristics of key aquatic breeding habitats of these mosquitoes, and (c) the susceptibility of the mosquitoes to insecticides commonly used for vector control.

The main finding was that, larval indices (container index (CI), house index (HI) and breteaux index (BI)) are high enough to signal significant risk of *Aedes*-borne diseases in the area. In the rainy season in particular, house and container indices in all wards exceeded the threshold value of 5.0, specified by WHO for actionable arboviral infections risk (Organization, 1971; World Health Organization, 1971; World Health Organization, 2016). Dry season risk was however confined to fewer wards though not completely absent from the rest of the wards. Immature *Ae. aegypti* infestation varied between wards and seasons, but remained significant even in dry season. This is expected since *Aedes* mosquitoes typically breed in man-made containers not fully dependent on rainfall. Besides, the vectors have fewer options of breeding sites in dry season hence elevating container level of infestation with immature *Ae. aegypti* (Table 2). On the contrary, aquatic habitats were relatively large in number during the rainy season, resulting in lower positivity rates (Table 2). This higher level of container infestation in the dry season concur with the study conducted in northern regions of Ghana which showed that, indices in the dry season was aggravated by poor water supply system in the area. As a result, facilitated the storing of water in pots and barrels for a period enough to bred *Aedes* mosquitoes (Appawu *et al*., 2010).

We noted that *Ae. aegypti* prefers breeding in clean and stagnant waters. Similar to other studies (Getachew *et al*., 2015; Mathias *et al*., 2017; Simard *et al*., 2005). Common habitats for *Ae. aegypti* were used tires, clay pots, flower pots, containers, coconut tree holes, pits, and on rare occasion disposed shoes, cooking pans, broken grasses and tarpaulins. Majority of these habitats were easy to discard, indicating an opportunity for proper waste management and environmental management as effective options for *Aedes* control, studies, tires in particular serve as important breeding sites for *Ae. aegypti* because they can hold water for long periods even in dry season (Getachew *et al*., 2015; Mboera *et al*., 2016; Ngugi *et al*., 2017). The multiple applications of used tires in the area will however complicate efforts to effectively dispose of the tires. For example, we observed that people use these tires as make-shift chairs, for playing by kids, for planting trees (residents believed that tires prevent plant pests) and for vehicle repairs.

A major natural breeding site in the area was coconut trees, which had artificial holes created for climbing during the coconut harvesting period. These holes served as perfect breeding sites for *Ae. aegypti* mosquitoes. We recommend that coconut tree holes be filled with sands to prevent rainwater from stagnating (World Health Organization, 1997). Clay pots were also common in Katindiuka and Lipangalala wards where they were mostly used for collecting rainwater for various domestic purposes. Unfortunately, residents did not know these pots bred mosquitoes. We also observed rare habitats such as disposed coconut shells, broken glass, animal feeding containers, tarpaulins and discarded plastic shoes which produced high larval abundance (larvae/dipper). Higher larval abundance was influenced by the size of the habitats and the volume of water present breeding sites.

During the data collection period, we raised awareness in surrounding communities about mosquito breeding behaviors and diseases they transmit. This led to a better understanding for them, and greater engagement of the communities in our work. Some breeding sites observed during the first visit were not there during subsequent visit as people became aware of the risks and hence proactively removed or covered potential habitats. This observation highlights the potential of educating communities about *Ae. aegypti* mosquito habitat sources and participatory control efforts. In Tanzania, the government is already implementing monthly clean-up campaigns,

which could be leveraged to achieve such gains. Moreover, efforts to reduce mosquito population can prioritize areas identified with higher risk.

In the adult surveys, the GAT trap collected more mosquitoes in dry season compared to rainy season. This was probably because in the rainy season there are many breeding options for *Aedes* mosquitoes, potentially outcompeting the GAT trap. The case was different for larval surveys whereby high number of larvae were obtained in the rainy season. Majority of larvae were collected in Ifakara town and Viwanja sitini while adult mosquitoes were mostly collected from Katindiuka.

Mosquito sizes play an important role in overall vector competence, vectorial capacity and ability to disseminate viruses (Alto *et al*., 2008; Paulson & Hawley, 1991). Smaller mosquitoes tend to have high contacts with hosts as they need more frequent blood meals than bigger mosquitoes, a phenomenon which could increase transmission (Alto *et al*., 2008). On the other hand, bigger mosquitoes have been demonstrated to be more resistant toward insecticides (Oliver & Brooke, 2013). Here, the wing length measurements for *Ae. aegypti* was done as previously documented by Nasci (1986), and showed a range of 1.9 mm to 3.5 mm. We also observed differences between administrative wards, though the extent to which such variations affect pathogen spread remains to be determined.

Lastly, we assessed how *Ae. aegypti* mosquitoes in the area would responds to control by commonly available insecticides. Fortunately, this study showed that the mosquito populations here are still generally susceptible to most insecticide classes except for bendiocarb against which there was resistance during the rainy season. Since this study is the first in the area of its kind, there are no immediate comparisons for the resistance profile. However, in studies done in Dar es salaam, Peru and Burkina Faso, resistance to pyrethroids and organophosphate was marked (Mathias *et al*., 2017; Pinto *et al*., 2019; Sombié *et al*., 2019). In our study, we have also observed notable spatial and seasonal variation toward Bendiocarb. Similar observation was previously documented for *Anopheles arabiensis* and *Culex pipiens* in south-eastern Tanzania (Matowo *et al*., 2019, 2017). Reduced susceptibility to pyrethroids observed in some of our assays, and the resistance seen against bendiocarb in the rainy season are however signs that we must remain vigilant as insecticide resistance could rapidly spread among the vector populations once active

control programs begin. This would therefore mean that environmental management, including larval habitats search and removal, should be an important component of any anti-*Aedes* campaigns. As most habitats are those that can be discarded, combinations of insecticidal and non-insecticidal approaches would likely be effective.

Though the main objectives were successfully completed in due time, this study also had the following two main limitations. First, the present study used a small number of Gravid *Aedes* Trap (GAT) for collecting adult mosquitoes (only 20 traps for all 170 grids). Therefore, the distribution may not have been captured effectively. However, since we rotated the traps between grids, over the entire collection season, the sampling gaps were considerably reduced. Second, larvae and pupae were only collected in the selected grids, i.e. search (34 grids per ward). These included grids with human occupations or buildings, but were not of the same area as entire study area (Fig. 2). As a result, it is possible that overall densities and distribution were slightly underestimated.

Lastly, we adopted WHO standard doses specified for *Anopheles* mosquitoes, as we still do not have a comprehensive guideline for *Aedes* mosquitoes. However, some of these insecticides, such as pirimiphos methyl, permethrin and deltamethrin already have diagnostic concentrations specific for *Aedes* mosquitoes. It is possible that if the right concentration were used, the results might have been different. For instance, results for permethrin (0.75%) demonstrated susceptibility toward standard concentration for *Anopheles*, which is three times the standard concentration for *Aedes* (0.25%). This means *Aedes* mosquitoes might be resistant toward its own concentration, but susceptible toward *Anopheles* concentration. Future studies will investigate these differences.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

This is the first study on the ecology and insecticide susceptibility of *Aedes* mosquitoes in this area, and will provide a basis for future evaluation of its role in pathogen transmission, as well as options for its control. Infestation levels observed indicate that immediate action should be taken to prevent outbreaks. The larval indices (container index, house index and breteaux index) are high enough to signal significant risk of *Aedes*-borne diseases in the area. Fortunately, the *Ae. aegypti* in the area are still susceptible to majority of insecticides used in public health, indicating available opportunities to include insecticides in the control programs. Since most habitats were those that can be discarded, integrating concepts of environmental management, insecticide use and community engagement could yield significant progress. While used tires, discarded containers and flower pots are key habitats for *Aedes* in the area, this study also identified coconut harvesting as an important risk factor, and the associated tree-holes as vital targets for *Aedes* control.

5.2 Recommendations

Base on the findings and observation from this study, I strongly recommend the following;

(i) Since most habitats were those that can be discarded, integrating concepts of environmental management, insecticide use and community engagement could yield significant progress.

(ii) Similar studies should be done in other small towns and secondary cities to establish the risk.

(iii) Given the high level of risk observed, authorities should embark on control of *Aedes* mosquitoes to stem any potential infections.

(iv) Insecticide susceptibility experiments studies should incorporate appropriate specific guidelines and right concentration for the specific species.

(v) Additional surveys, should be done on human populations, e.g. in hospitals to ascertain any risk to human populations.

(vi) Control measures should involve communities, so as to tackle challenges such as coconut tree holes which are both beneficial to communities and are dangerous sources of *Aedes*.

(vii) Future studies should consider to use as many traps as possible so get the full picture of the mosquito's distribution.

(viii) Future studies should consider all the grids occupied by human habitations and building to obtain wide coverage of the area.

(ix) To fully understand the seasonal variation, future studies should consider to investigate the densities of mosquitoes and resistance profile throughout the year.

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APPENDICES

Appendix 1: Characterization of *Aedes* breeding habitat site surveys

Field Form (To be filled in full at the breeding site)

Grid ID: …… Names of fieldworker: …….

Ward ID: ……. DATE: …… Time …….

Latitude …… Longitude …… Elevation …….
Habitat ID: ……

1. Location
    a) Indoor
    b) Outdoor
2. Water movement
    a) Stagnant
    b) Slow moving
    c) Fast moving
3. Source of water
    a) Rain water
    b) Domestic water
4. Water status
    a) Clear
    b) Colored
    c) Polluted
5. Habitat type
    a) Tree holes
    b) Flower pot
    c) Used tires
    d) Bucket
    e) Disposed containers
    f) Clay pot
    g) Pit
    Others……
6. Habitat size
    a) Large
    b) Medium
    c) Small
7. Water type
    a) Permanent include sewers, wells e.t.c
    b) Temporary include disposed container
8. Shades over habitat
    a) None
    b) Partial
    c) Full
9. Source of shades
    a) Vegetation
    b) House
    c) Habitat itself
    d) Roof
    Others ……
10. Vegetation quantity around habitat
    a) Scarce
    b) Moderate
    c) Abundant
11. Environment features (around water habitat)
    a) Grazing
    b) Cultivated field
    c) Swamp area
12. Social activities around the habitat
    a) School/ College
    b) Market places
    c) Football ground
    d) Residential area
    e) Garage
13. Additional observed information

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……

Larval sampling

Larvae present? *Anopheline* Y / N
Culex Y / N
Aedes Y / N

Number of dips ……

Species	Larvae	Pupae
Culex		
Aedes		

D/N	*Aedes* (350ml)	Culex (350ml)	*Aedes* (70ml)	Culex (70ml)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Grid ID	House inspected	House infested (*Aedes* positive)	Container inspected	Container infested (Container positive)

Appendix 2: Form for recording insecticide susceptibility of *Aedes aegypti* mosquitoes

a. Susceptibility testing information

Ward code: ……. ☐ Test number: ☐ Date: ☐

Investigator name: …….

Area information

Country: …… Province…….……...

District……. Ward…….

GPS position UTM_X ☐ GPS position UTM_X ☐

Sample information

Species tested: ……... Species control: …….

Sex: ……... Age: ……...

Collection method

Human landing indoor	☐	Resting nightly indoor	☐	Resting morning indoor	☐
Cattle collect	☐	Human landing outdoor	☐	Resting nightly outdoor	☐
Other: Specify ……	☐	Larval collection	☐	Progeny F1	☐
Colony	☐	Name of colony strain……			

Physiological stage

Non-blood fed ☐ Blood fed ☐ Semi-gravid ☐ Gravid ☐

Test insecticide information

Insecticide tested: ……. Date of expiry: ……/……. / ……

Impregnated paper by: ……. Date box first open: ……/……. / ……

Concentration: ……. Number of times this paper is used …….

Test conditions

	Exposure period: Start	After 12 h	End test
Temperature °C	☐☐☐	☐☐☐	☐☐☐
Relative humidity (%)	☐☐	☐☐	☐☐

b. Test results: Period of exposure (min)……...

No. exposed	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Control 1		Control 2	
Number of knocked down (KD) mosquitoes after exposure for min												
	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Control 1		Control 2	
	Time	No. KD	Time	No. KD	Time	No. KD	Time	No. KD	Time	No. KD	Time	No. KD
START												
10 min												
15 min												
20 min												
30 min												
40 min												
50 min												
60 min												

c. Number of dead/ alive mosquitoes at the end of holding period

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Control 1	Control 2
No. dead						
No. alive						